Lab # _____ DNA Fingerprinting

Lab Instructor _____ Name _____
Date _____ Period _____

Objective: To study DNA fingerprinting

*** *Use full sentences when answering all questions.* ***

Background

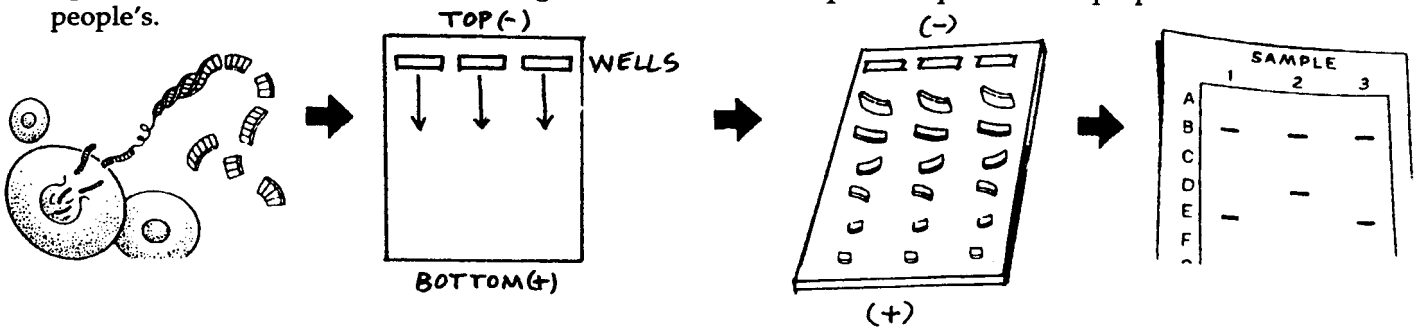
Except for identical twins, every person has a DNA profile that is unique. A technique called DNA fingerprinting can be used to compare the small differences among DNA sequences of different individuals. To create a DNA fingerprint, a sample of tissue such as hair root cells or cells from inside the cheeks must be obtained from the person. Next, the DNA from the tissue sample is extracted. Then the very long molecules of DNA are cut up using restriction enzymes.

Restriction enzymes are enzymes that come from bacterial cells that can cut double-stranded DNA into fragments. There are about three hundred different restriction enzymes, and each cuts at a specific base sequence called the recognition sequence. For example, the restriction enzyme RsaI cuts DNA at the base sequence GTAC / CATG and the restriction enzyme EcoRI has the recognition sequence GAATTC / CTTAAG

If we mix a person's DNA sample and a particular restriction enzyme in a test tube, the restriction enzyme will cut the DNA wherever the recognition sequence occurs, resulting in various sized DNA fragments. When the restriction enzyme cuts the DNA, we say the DNA has been digested.

Gel electrophoresis is a laboratory technique that allows us to separate the DNA fragments of different lengths and then visualize them. We can put our restriction-enzyme-digested DNA sample, which contains DNA of various lengths, into a well of a gel made of agarose. Because the phosphates of DNA are negatively charged, when our sample in the gel is subjected to an electric field, the DNA fragments will migrate (move) toward the positive pole. The small DNA fragments will migrate faster than the long ones, so that after gel electrophoresis, the different DNA fragments will be sorted by size. The longest fragments will be near the top of the gel, and the shortest fragments will be near the bottom.

Differences in DNA sequence between two people will cause their DNA to be cut in different places, and this will result in different sized fragments between the two people. After gel electrophoresis, we can see a person's different unique restriction fragments, and we can compare one person's unique pattern with other people's.



DNA is cut | **Gel with wells** | **Gel Electrophoresis** | **Visualize the DNA fragments**

PRE-LAB

1. Why is it possible to use any tissue from the body to obtain a DNA sample?

2. What is an enzyme? What type of biological molecule are all enzymes made of?

3. What do restriction enzymes do?

4. From its name, what type of molecule is agarose?

5. What causes the DNA fragments to migrate across the gel?

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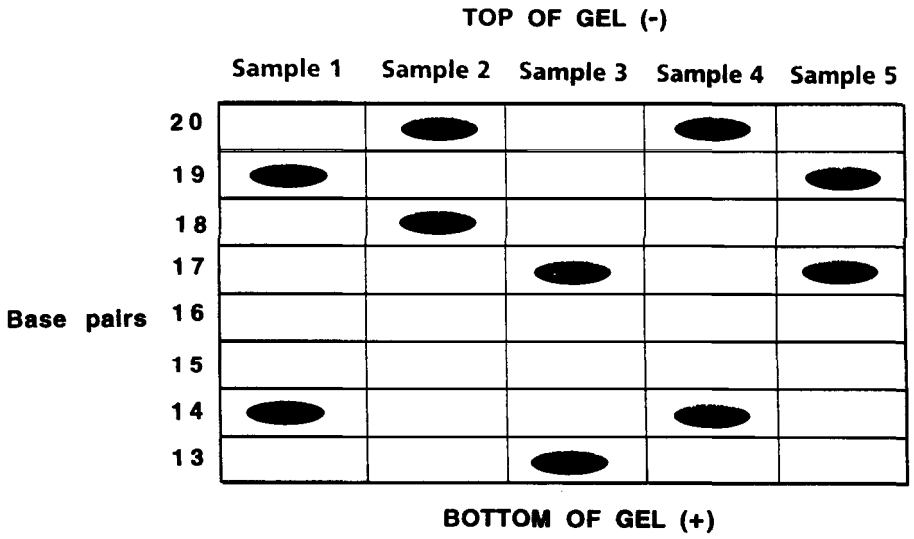
6. At a local hospital, three new-born babies were brought to the nursery at the same time. The nurses want to be sure that the babies are given to the correct parents. A restriction enzyme analysis is performed on blood samples from the babies and the parents. Below is the resulting electrophoretic gel for the three babies and one set of parents.

Sample 1: Baby 1
Sample 2: Baby 2
Sample 3: Baby 3
Sample 4: Father
Sample 5: Mother



Keeping in mind that individuals receive half their DNA from their mother and half from their father, use the gel to determine which baby belongs to the parents. Explain.

LAB----------

Materials

1. Five DNA sequences from blood samples related to a murder. One sample is from the victim, one from the crime scene, and three from three different suspects.
2. A blank form representing an electrophoresis gel.
3. Scissors
4. glue or tape

Procedures

1. For each DNA molecule, find the recognition sequence for the restriction enzyme RsaI, which is

   GTAC
   CATG

For each DNA sample, there may be more than one occurrences of the recognition sequence.

2. Each time you find a recognition sequence, draw a line through it, indicating where the DNA will be cut by RsaI, as shown on the page with the DNA sequences.
3. *Count the number of base pairs in each resulting fragment.*
4. Cut out each DNA molecule, cut it into the different size RsaI fragments, and adhere each fragment to the electrophoresis gel form under its correct well and at the correct fragment length. If the fragment is too long to fit in the space, fold it up so that it will fit.

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Observations

1. Using the finished gel, record the different size fragments from each sample.

Well 1: _____

Well 2: _____

Well 3: _____

Well 4: _____

Well 5: _____

POST-LAB/CONCLUSIONS

1. Based on our analysis, which suspects are no longer under suspicion, and which suspect will be arrested for the murder? Why?

2. Why was the DNA from the victim in the crime included in the experiment?

3. Does our analysis prove that one of the suspects committed the murder? Why or why not?

4. Using the words chromosome and fertilization, how would you explain the results of the gel in Pre-lab Question 6?

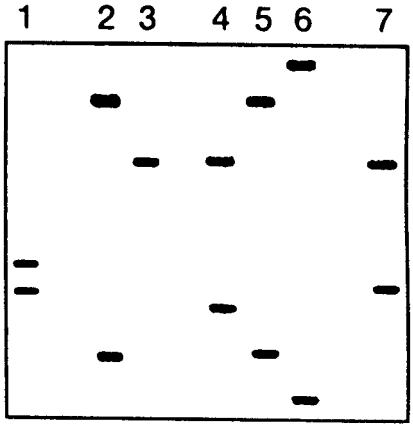
5. This photograph shows a restriction analysis used to determine whether one of four suspects was involved in a crime.

a) Which suspect's DNA fingerprint matches the DNA in the blood found at the crime scene?

b) Why was the DNA from the victim in the crime included in the experiment?

c) Why was a control included?

d) How likely is it that blood found at the crime scene belongs to one of the suspects?



KEY

1 Control	4 Suspect 1
2 Blood at crime scene	5 Suspect 2
3 Victim	6 Suspect 3
	7 Suspect 4

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Rsa I recognition sequence: GTAC
(Cuts across both strands) CATG

For example, cutting this sequence with RsaI will result in 2 fragments, one that is 4 base pairs long, and one that is 5 base pairs long.

CAGTACTGC
GTCATGACG

Well 1: Blood found at the crime scene

AATAGACGTACACTTAGGACTACAAAGTACCCCCTAGGACATGGGTACATGTACTCCAGTACTGATTCACTCGGTACCGTGGCACAGTAAAACGAT
TTATCTGCATGTGAATCCTGATGTTTCATGGGGGATCCTGTACCCATGTACATGAGGTCATGACTAAGTGACCCATGGCACCGTGTCATTTTGCTA

Well 2: Victim

CCTGTACGGTCAGGCTAAGCTAGCCGTACTACTGCTAGCTAGCTGTACATAGCAAATCGGGATGGTACGTACTGATCGATCATGGTACACTTTGGT
GGACATGCCAGTCCGATTCGATCGGCATGATGACGATCGATCGACATGTATCGTTTAGCCCTACCATGCATGACTAGCTAGTACCATGTGAAACCA

Well 3: Suspect 1

CCAATGTTGGTAACGTATGTACGCAGTACAAACGGTGGTACTGTCAGTGTACGTAACAAAACGTGGGTACTCAACGTACGTCTCGGCGGGTACCAT
GGTTACAACCATTGCATACATGCGTCATGTTTGCCACCATGACAGTCACATGCATTGTTTTGCACCCATGAGTTGCATGCAGAGCCGCCCATGGTA

Well 4: Suspect 2

CCCACGGATGTTGTACAGTGCAGAGTACAAATTGCTGACTGGTGGTACCAAGTGTACGTAATGCGCGTACACTTGTGTACTCTCAAGTCCTGTGGG
GGGTGCCTACAACATGTCACGTCTCATGTTTAACGACTGACCACCATGGTTCACATGCATTACGCGCATGTGAACACATGAGAGTTCAGGACACCC

Well 5: Suspect 3

AATAGACGTACACTTAGGACTACAAAGTACCCCCTAGGACATGGGTACATGTACTCCAGTACTGATTCACTCGGTACCGTGGCACAGTAAAACGAT
TTATCTGCATGTGAATCCTGATGTTTCATGGGGGATCCTGTACCCATGTACATGAGGTCATGACTAAGTGACCCATGGCACCGTGTCATTTTGCTA

TOP OF GEL (-)

	Blood from crime scene WELL 1	Blood from victim WELL 2	Blood from Suspect 1 WELL 3	Blood from Suspect 2 WELL 4	Blood from Suspect 3 WELL 5
22 base pairs					
21 base pairs					
20 base pairs					
19 base pairs					
18 base pairs					
17 base pairs					
16 base pairs					
15 base pairs					
14 base pairs					
13 base pairs					
12 base pairs					
11 base pairs					
10 base pairs					
9 base pairs					
8 base pairs					
7 base pairs					
6 base pairs					
5 base pairs					
4 base pairs					
3 base pairs					
2 base pairs					
1 base pair					

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BOTTOM OF GEL (+)